

**CLAIM AMENDMENTS**

1.-25. (cancelled)

26. (currently amended, previously once amended) A method for detecting HCV nucleic acid in a biological sample ~~comprising~~ consisting of the steps of:  
having a diagnostic kit comprising an amplification component and a detection component, the amplification component comprising a forward primer comprising SEQ. ID. NO. 1 and a reverse primer comprising SEQ. ID. NO. 2, the detection component comprising a probe comprising SEQ. ID. NO. 3,

extracting HCV nucleic acid from a biological sample;

amplifying the HCV nucleic acid using ~~a first primer having the sequence~~

5'-gcagaaagcgtctagccatggcgt-3' [SEQ. ID. NO. 1]

~~a second primer having the sequence~~

5'-ctcgcaagcaccctatcaggcagt-3' [SEQ. ID. NO. 2]

and an the amplification component ~~consisting essentially of about 100 to about 200  $\mu$ M of deoxyribonucleoside triphosphate; about 1 unit to about 2.5 units of Taq polymerase; about 1.5 to about 2.5 mM  $MgCl_2$ ; and an amplification buffer having 10 mM Tris HCl (pH 8.3) and 500mM KCl;~~

and detecting the presence of the HCV nucleic acid using an oligonucleotide probe having comprising the sequence:

5'-gtcgtgcagcctcaggacc-3' [SEQ. ID. NO. 3]

the detection component.

wherein the presence of HCV in the biological sample is confirmed by the color of the detection component.

27. (original) The method according to claim 26, wherein the biological sample is selected from the group consisting of: serum, plasma, and combinations thereof.

28. (currently amended) The method according to claim 26, wherein the ~~first~~ forward and ~~second~~ reverse amplification primers ~~have a label~~ are fluorescein-labeled at their respective 5' ends.

29. (cancelled)

30. (currently amended) The method according to claim 26, wherein the step of amplifying the HCV nucleic acid includes:

denaturing the HCV nucleic acid to produce denatured HCV nucleic acid;  
annealing the ~~first~~ forward and ~~second~~ reverse amplification primers to the denatured HCV nucleic acid to produce primed HCV nucleic acid; and  
extending the primed HCV nucleic acid using a thermostable DNA dependent DNA polymerase in the presence of a deoxyribonucleoside triphosphate.

31. (original) The method according to claim 30, wherein the DNA dependent DNA polymerase is Taq polymerase present in an amount of about 1 Unit to about 2.5 Units.

32. (previously once amended) The method according to claim 30, wherein the deoxyribonucleoside triphosphate is selected from the group consisting of: dATP, dCTP, 5MedCTP, dGTP, dTTP, dUTP, and combinations thereof.

33. (original) The method according to claim 26, wherein the step of detecting the HCV nucleic acid includes:

binding the HCV nucleic acid with the oligonucleotide probe attached to a solid medium to form immobilized HCV nucleic acid;

binding the immobilized HCV nucleic acid with a conjugate; and

adding a substrate that is adapted to change color in the presence of an enzyme on the conjugate,

whereby a change of the color of the substrate indicates the presence of HCV nucleic acid.

34. (previously once amended) The method according to claim 33, wherein the HCV nucleic acid is labeled with fluorescein, and wherein the ~~detectable marker~~ conjugate is an anti-fluorescein /horseradish peroxidase conjugate in an amount of about 1 unit to about 4 units.

35. (original) The method according to claim 33, wherein the substrate comprises hydrogen peroxide and 3,3',5,5'-Tetra methyl benzidine Dihydrochloride.

36. (previously once amended) The method according to claim 33, wherein the substrate is present in a volume of about 100  $\mu$ L.

37. (original) The method according to claim 33, further comprising the step of reading a change of the color of the substrate with a colorimetric plate reader.

38. (currently amended, previously added) A method for detecting HCV nucleic acid in a biological sample ~~comprising~~ consisting of the steps of:

having a diagnostic kit comprising a fluorescein-labeled forward oligonucleotide primer comprising SEQ. ID. NO. 1, fluorescein-labeled reverse oligonucleotide primer comprising SEQ. ID. NO. 2, and an oligonucleotide probe comprising SEQ. ID. NO. 3;

extracting HCV nucleic acid from a biological sample with a chaotropic agent to produce extracted HCV nucleic acid;

reverse transcribing the extracted HCV nucleic acid to produce DNA using avian myeloblastosis virus reverse transcriptase in a buffer consisting essentially of 50 mM Tris.HCl (pH 8.3), 6 mM  $MgCl_2$ , 40 mM KCl, 4 mM dithiothreitol, and dNTP's in a concentration of 100-200  $\mu$ M;

producing fluorescein labeled amplimers by amplifying the DNA using an amplification component consisting essentially of: about 100 to about 200  $\mu$ M each of dATP, dCTP, dTTP and dGTP; about 1 unit to about 2.5 units of Taq polymerase; about 10 to about 100 pM of a ~~first~~ the forward oligonucleotide primer comprising (SEQ. ID. NO. 1) ~~labeled with fluorescein~~ fluorescein; about 10 to about 100 pM of a ~~second~~ the reverse oligonucleotide primer comprising (SEQ. ID. NO. 2) ~~labeled with fluorescein~~

fluorescein; about 1.5 to about 2.5 mM  $MgCl_2$ ; and an amplification buffer having  
comprising 10 mM Tris HCl ( pH 8.3) and 500mM KCl;

denaturing the fluorescein labeled amplimers;

providing ~~an~~ the oligonucleotide probe comprising (SEQ. ID. NO. 3) immobilized  
on a solid medium in a microwell;

adding the denatured fluorescein labeled amplimers to the microwell, thereby  
immobilizing the denatured fluorescein labeled amplimers on the solid medium;

adding anti-fluorescein/horseradish peroxidase conjugate to the microwell,  
thereby binding the anti-fluorescein/horseradish peroxidase conjugate to immobilized  
denatured fluorescein labeled amplimers; and

adding a detection solution comprising TMB to the microwell to produce a  
TMB/horseradish peroxidase reaction,

whereby the presence of HCV in the biological sample is confirmed by the color  
of the detection solution after the TMB/horseradish peroxidase reaction is stopped.--